

INTERPOPULATIONAL VARIATION OF BLOOD PROTEINS IN PIKA (*OCHOTONA PRINCEPS SAXATILIS*)

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ABSTRACT.— Studies were undertaken to examine the degree of divergence in four populations of pika in Colorado. Separation of blood proteins was accomplished utilizing acrylamide-gel disc electrophoresis. Separate analyses of variance were carried out for the relative mobilities of two of the transferrins (designated $RM\beta_1$ and $RM\beta_2$), the mean relative distance difference of the two transferrins (DM), and the density of the most prominent albumin (α_1) and transferrin (β_2) bands. Although the four populations were characterized by a general similarity of the gel patterns, both interpopulational and sexual variations were observed. Variations between the sexes had to do with the amounts of protein, not with differences in protein mobility. The only significant populational differences were found in reference to density and DM. The interpopulational differences may serve as an indicator of populational divergence that has not been indicated by standard morphological characters. The significant patterns of variation observed in the blood proteins of the four populations studied may reflect a lack of gene flow between isolated populations.

North American pika are boreal mammals that occur in "island" populations on mountains throughout much of western North America. Pika are restricted to a narrowly defined habitat (the talus zone), and movement between neighboring populations is likely to be quite limited. Smith (1974) has estimated, for example, that distances greater than 300 m appeared to pose difficult barriers to dispersing juveniles.

The extent of genetic isolation among different populations is largely unknown, and studies aimed at determining geographic variation in pika are necessary. The subspecies of *Ochotona princeps* were revised by Howell (1924), and a synopsis is provided by Hall (1951). A total of 35 subspecies are currently recognized. The subspecies do differ in their cranial dimensions, but previous reports have indicated that there is little divergence in pika populations on the basis of size morphology.

The present study was designed to examine variations in blood proteins in four populations of *Ochotona princeps saxatilis* in Colorado.

The interpretation of electrophoretic mobility of various blood constituents as a

means of discovering differences between separate populations has received much discussion and criticism over the past few years. However, the fact that most of these substances are partly protein, thus bearing a close translational relationship to the gene itself, points up at least hypothetically the value of such investigations.

METHODS

Collecting Localities: A total of 41 pika were collected from four localities in Colorado. The areas were Evans and Goliath in Clear Creek County, Audubon in Boulder County, and Crown Point in Larimer County. The ranges of elevations over which animals were collected at each site were 3,902–4,085 m, 3,537–3,659 m, 3,598–3,841 m, and 3,354–3,506 m, respectively.

Blood Samples: Animals were shot and blood was withdrawn from the heart usually within three minutes. Heparinized 1.4–1.6 mm micro-hematocrit capillary tubes were filled to within one-half inch of one end, and were subsequently made airtight at both ends with plastic Crito-caps. All sealed hematocrit tubes were placed in glass vials,

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packed in snow, and shielded from sunlight during transport to the laboratory. Immediately upon arrival in the laboratory, hematocrit tubes were centrifuged at 11,500 rpm using an IEC microcapillary centrifuge. The tubes were broken at the cell-plasma interface, and the plasma was frozen until electrophoretic determinations were made.

Electrophoresis: Electrophoresis of plasma proteins from 41 animals was accomplished using the acrylamide-gel disc technique (7 percent acrylamide) described by Smith (1968).

The electrophoretic chamber (Buchler Instruments) held a total of 12 gel tubes. Current was applied initially for 12 minutes at 1.5 milli-amperes/gel in a tris-glycine buffer at pH 9.5. Following this initial warm-up period, gels were run at 4.0 milli-amperes/gel for 30 minutes. A gelman six-volt power source was used for all separations. No spacer or sample gel was employed, and 20 μ l of plasma was mixed with 40 μ l of 50 percent sucrose and was placed directly on the running gel. Tubes containing purified bovine albumin were run as controls.

Following separation, gels were stained for 45 minutes in aniline blue-black. Destaining required approximately five days and was accomplished in a series of three 7 percent acetic acid baths. Whole gels were analyzed densitometrically using a Densicord recording electrophoresis densitometer (Photovolt Corporation). An integrator (Integragraph Model 49) automatically measured

the areas under the densitometric curves. Analysis of densitometric curves followed the procedures outlined by Dalby and Lillevic (1969).

RESULTS AND DISCUSSION

Table 1 summarizes mean values resulting from measurements of densitometric tracings of the stained acrylamide gels. Because no tests were run to confirm the chemical identity of the protein bands, $RM\beta_1$ and $RM\beta_2$ refer only to relative mobilities of what are presumed to be the same two bands in the transferrin range. Likewise, α_1 and β_1 are measurements of the height of the densitometric curve of the most prominent presumed albumin and transferrin band respectively. The parameter DM represents

$$\frac{\Sigma(RM\beta_2 - RM\beta_1)}{n}$$

for each of the eight sample groups investigated.

Analyses of variance were run on $RM\beta_1$, $RM\beta_2$, albumin and transferrin heights, and the mean relative distance (DM). Patterns of statistically valid sexual variation were seen in regard to the height of both the α and β bands; sexual differences in $RM\beta_1$, $RM\beta_2$, and DM were not significant. Statistically significant differences among geographical collecting areas were found only in reference to β_1 height and DM. None of

TABLE 1. Electrophoretic data: Means \pm Standard Deviations.

Area	Sex	Sample Size	$RM\beta_1$	$RM\beta_2$	α_1	β_1	DM
Evans	Males	5	0.569 \pm 0.004	0.717 \pm 0.005	21.800 \pm 0.447	10.000 \pm 2.121	0.147 \pm 0.001
Evans	Females	10	0.575 \pm 0.015	0.718 \pm 0.014	21.700 \pm 0.483	10.300 \pm 2.907	0.143 \pm 0.010
Audubon	Males	6	0.576 \pm 0.006	0.698 \pm 0.006	21.166 \pm 0.408	6.166 \pm 1.602	0.122 \pm 0.003
Audubon	Females	3	0.575 \pm 0.007	0.716 \pm 0.003	21.666 \pm 0.577	7.333 \pm 1.154	0.140 \pm 0.005
Goliath	Males	3	0.569 \pm 0.010	0.717 \pm 0.008	21.666 \pm 0.577	7.666 \pm 0.577	0.148 \pm 0.002
Goliath	Females	7	0.568 \pm 0.007	0.721 \pm 0.011	21.857 \pm 0.377	11.285 \pm 3.039	0.153 \pm 0.008
Crown	Males	4	0.565 \pm 0.006	0.704 \pm 0.024	21.000 \pm 0.816	5.750 \pm 1.500	0.139 \pm 0.023
Crown	Females	3	0.569 \pm 0.006	0.702 \pm 0.039	22.000 \pm 0.000	8.666 \pm 1.527	0.132 \pm 0.036

the Area X sex interactions were significant.

The main objective in regard to examination of patterns of electrophoretic plasma protein migration was to uncover possible interpopulational variations of a striking nature. If these variations were to exist, they perhaps might be an indication of a stable phenotypic characteristic, which, when viewed with phenotypic characters of a morphological type, might indicate a high or low degree of populational divergence.

That this divergence might be expected is based on the relative altitudinal isolation of pika populations in Colorado. The fact that high talus regions are generally separated by non-talus areas and the general reluctance of the pika to leave the talus habitat seems to preclude the necessity of distance as a block to gene flow. This does not mean to suggest that gene flow is the salient factor causing population divergence. It is possible that even in the absence of continued gene exchange, these animals have evolved in parallel fashion due to the relative similarity of selective pressures.

Crown Point and Mount Audubon are widely separated from each other as well as from Mount Evans and Mount Goliath. There would seem, in these instances, to be a reduced amount of reproductive activity between populations. Because they are separated by a distance of only about five miles, Evans and Goliath might be treated as a single population. It is possible, however, that gene flow even between these two areas has been minimal or non-existent.

Although divergence of three of these populations might be expected on the basis of restricted gene exchange, the present classifications of North American pikas are based entirely on morphological characters which, with the exception of pelage color, seem to show little variation (Broadbooks 1965). It was deemed worthwhile, then, to examine plasma protein migration patterns as a more precise indication of possible populational differences.

In interpreting any variation found in a study of this nature, one must resist the urge to attribute them all to stable genotypic characteristics. Indeed, more information is needed from breeding experiments

and from expanded analysis of individual protein components before a more confident stand can be taken in regard to the genotypic stability of a difference. There is no question that the literature warns against over-zealous interpretation of any measured protein variations. It might be prudent, therefore, to approach interpretation of data of this type from the standpoint of similarities in migration patterns.

Although patterns of both interpopulational and sexual variation are present, there is a general similarity of the gel patterns from the four areas. In addition to the obvious height differences in some curves, the presence of a dense band very close to the origin in some samples from both sexes from Mount Evans and from Mount Goliath are the most noticeable differences in an otherwise generally common pattern.

The presence of significant sexual variation is not surprising, as was pointed out by Moore (1945). Female animals do seem to have denser β_1 and α_1 bands than do males. It is interesting that there seem to be differences only in the amount of a protein rather than the type of protein molecule, as there were no significant patterns of variation in protein mobility between sexes. The significant pattern of variation between locations in regard to the β_1 band is probably due to bias introduced by the larger number of female samples from some locations.

The significant differences in DM between geographic areas seem to indicate differential migration rates of the entire samples. The DM is, in fact, a measure of the overall linear variation in both the gels (and consequently the tracings), and it is precisely why the tracings do not superimpose exactly. These interpopulational differences seem to be real. That they are stable genetic characters cannot be determined from this analysis; they may, however, be an indicator of population divergence that cannot be measured in terms of standard morphological characters.

Although interpretation of electrophoretic plasma protein migration patterns should be approached with caution, such interpretation has the potential of uncovering

more subtle differences that may not be mirrored in standard taxonomic characters. At this point, one can only say that the significant patterns of variation found in regard to plasma protein mobility may support the possibility of a predicted lack of gene flow between isolated populations of pika.

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